THE RESPIRATION OF PUFFER FISH

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The mechanism of respiration of fishes which live in the sea offers an attractive and productive subject for study. The ocean is stable and uniform and therefore a favorable environment for living organisms. An abundant supply of oxygen is usually present. The hydrogen ion concentration varies only in a range which is close to the optimum for physiological processes, especially for the elimination of carbon dioxide. The temperature of the ocean as compared with freshwater and land conditions is relatively uniform. Moreover, sea water is similar in constitution to the internal fluids of marine organisms. Since most vertebrates have mechanisms for maintaining conditions within their bodies more or less constant and since fishes are the last of typically marine vertebrates to evolve, it seems important to study the factors which vary in sea water and which in some manner influence the respiratory exchange of gases between fishes and their surroundings.

Fishes breathe dissolved gases from water which they pump over their gills. The mechanism for external respiration consists in most fishes of rhythmical suction of water into the oral cavity and its subsequent expulsion through the gill clefts. During inspiration the mouth is opened and the oral cavity enlarged by the lateral expansion of its walls. When the oral cavity is closed the expiratory process begins. By the lateral contraction of the oral walls water is driven through the gill clefts and over the gill filaments. The branchial arches are spread apart during the expiratory phase, thus permitting all of the filaments to come into direct contact with the circulating water. The gas exchange between the blood and water takes place through the walls of the filaments.

Considering the general mechanics of external respiration as shown by fishes, several problems come to mind. How much water is pumped in a single respiratory cycle? How much of the dissolved oxygen is removed from sea water as it passes the gills? When an increased oxygen supply is required, which plays the more important rôle—an increase in the ability of the gills to absorb oxygen from the sea water; an increase in the volume of water pumped by a single respiratory cycle; or an increase in the number of respiratory cycles per unit time? Consideration is given to each of these possibilities in the following pages.

Three physico-chemical factors which may vary in the external medium and affect the equilibrium which the organism maintains in its internal environment are temperature, oxygen tension, hydrogen ion concentration (carbon dioxide tension and hydrogen ion concentration *per se*). By varying these factors in the investigations to be described, a means of studying certain phases of the general problem of respiration was found.

The most extensive studies bearing on the problems of fish respiration are those of Winterstein (1908). He used the fresh-water fish, *Leuciscus erythrophtalmus.* Fishes under observation were held fast by a clamp, while a constant stream of water of a known oxygen tension was passed over the gills by means of a thick canula fastened in the mouth of the fish. The amount of oxygen used up was determined. This is perhaps the simplest and most direct method that has been devised for the determination of the respiratory exchange in fishes. However, as Winterstein has pointed out, one must keep in mind that the fishes are breathing somewhat abnormally. When fishes have water forced over their gills, they may not respire in the same way as if they were pumping the water over the gills in the natural manner. He concludes from his experiments that the oxygen consumption is independent of oxygen tension of the surroundings within wide limits of magnitude, and that the utilization of oxygen is in inverse proportion to the flowing velocity. Henze (1910) has also shown that oxygen consumption in certain fishes is not influenced to any great extent by the oxygen tension of the surrounding water. His results are expressed in arbitrary values and are not particularly constant.

Gaarder (1918) has performed an interesting experiment on the fresh water carp. His paper is stimulating and thoughtful. However, it discusses only a few analyses and has the disadvantage that the gills were subjected to forced ventilation and therefore were perhaps not functioning naturally. Gaarder had the misfortune, it appears, of being quoted inaccurately, being said to conclude that oxygen consumption is within wide limits proportional to oxygen tension. Another author quotes him as believing that oxygen consumption is independent of oxygen tension. The writer understands Gaarder's conclusion to be that consumption is uninfluenced so long as the hemoglobin of the blood is not fully saturated; when oxygen and the oxygen tension of the physically dissolved oxygen is raised considerably, then oxygen consumption shows an increase.

Powers (1922, 1929) and Powers and Shipe (1928) have shown that carbon dioxide tension and pH have a pronounced effect on the respiration of fishes. Powers (1930) has given an excellent summary of the relation between pH and aquatic animals.

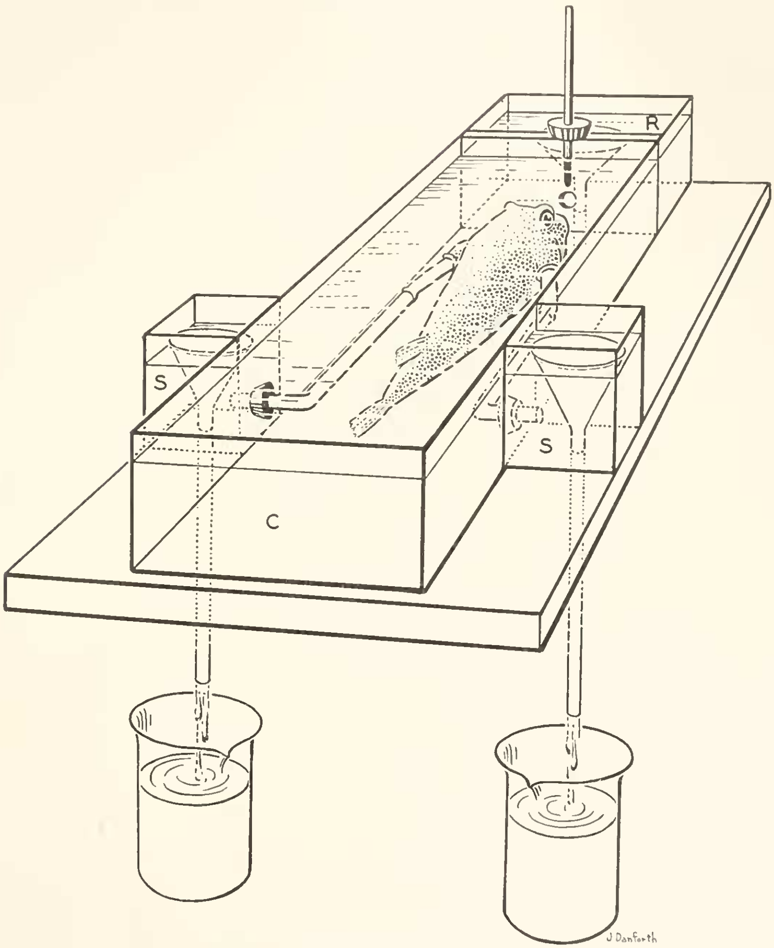

FIG. 1. Apparatus used for the determination of the influence of environmental factors on the respiration of puffer fishes.

METHODS

The puffer fish, *Spheroides maculatus* (Bloch and Schneider), was used in the writer's investigations because it was particularly adapted to such a study. The rounded shape of the opercular aperture, which is considerably reduced in size as compared with other fishes, makes this species especially advantageous. Glass tubes may be inserted through the opercular openings without apparent injury. All of the water pumped by the fish for respiratory purpose will then flow through the glass tubes and samples can be collected for analyses. Fishes carrying such tubes will lie quietly for hours apparently breathing normally, and will live in this condition for several weeks. Tubes were inserted in the opercular openings of puffers about three or four days previous to using them for the respiration experiments. Thus the animals became accustomed to breathing in such a manner.

The apparatus used in these experiments is shown in Fig. 1. The fish was submerged in a chamber (*c*), which had a capacity of 4 liters. A reservoir (*R*) to which flowing water was admitted and which also contained a funnel out of which all of the excess water flowed was connected to the chamber by a hole one inch in diameter. Two side compartments (*s*) were so arranged that they could be connected with the glass tubes inserted in the opercular opening of the fish. A funnel was placed in each side compartment at the same level as that in the reservoir (*R*). The height of the funnels in each case was adjusted so that before the fish was connected to the side compartments water entering the reservoir would flow out through the funnel in the reservoir but would not flow out through the funnels in the side compartments. Thus only a very slight exertion on the part of the fish was required to pump water from the chamber in which it was submerged to the funnels in the side compartments. Fishes were placed in the chamber so that their mouths were close to the hole leading from the reservoir. Thus a fresh flowing supply of water was always available. It was not found necessary to either anæsthetize these fishes or to clamp them. If they were left undisturbed by outside factors they would remain quiet for hours.

The quantity of water pumped per minute was measured by use of volumetric flasks placed under the funnels, and a stopwatch. The quantity of water pumped through the right and left gill chambers was taken separately. Analyses of the dissolved oxygen was made on the water before it entered the fish's mouth and after it had been pumped into the side compartments. The well known Winkler method as modified by Birge and Juday was employed. Care was taken not to expose the water to air in taking the samples.

In experiments where the influence of temperature was studied, water was cooled to the desired temperature by passing through coils in a constant temperature bath. A range of 10° C. was used since puffers do not readily adjust themselves to a lower temperature than 10°–11° C. or higher than 23°–24° C. The temperature range chosen for this experiment was 12°–22° C.

The hydrogen ion concentration of the water was measured by colorimetric means. Consequently the analyses do not represent a precise measurement or an absolute value since salt errors are introduced. No corrections have been made for salt errors. The pH determinations must be taken only as of relative values. In one type of experiment the hydrogen ion concentration of the water was controlled by the addition of carbon dioxide gas. In a second type hydrochloric acid was

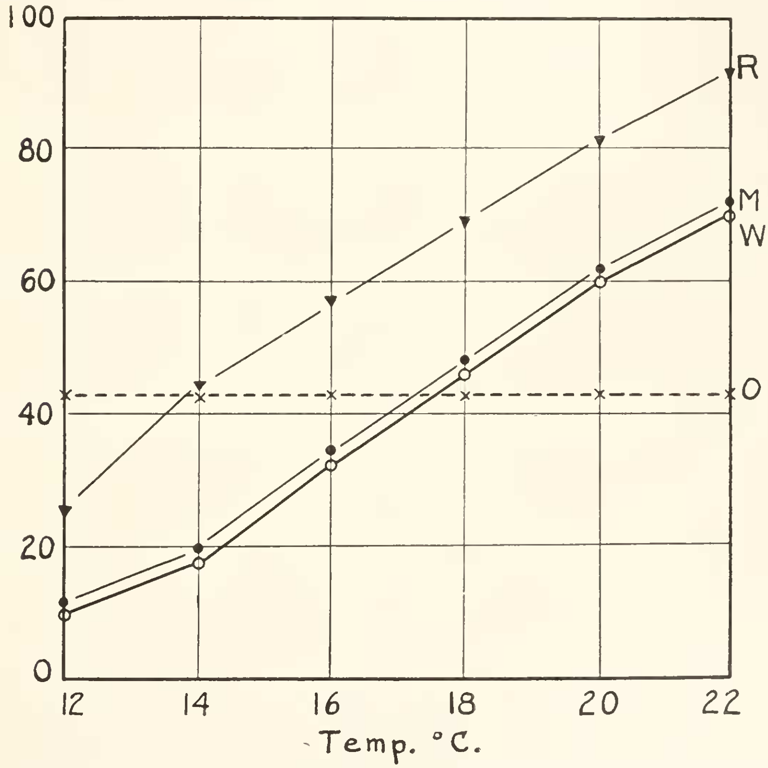

FIG. 2. Graph showing the influence of temperature on the respiration of puffer fishes. Respiratory rhythm (*R*) in respirations per minute; oxygen consumption (*M*) in cc. of oxygen per kilogram per hour; water pumped through branchial cavity (*W*) in deciliters per hour; percentage of dissolved oxygen (*O*) removed from the affluent water.

added to the water and the carbon dioxide formed was driven off by aëration.

Sea water of different oxygen tensions was procured by boiling and subsequent mixing with normal sea water. In this manner sea water of any desired oxygen tension could be obtained.

RESULTS

The results of the first experiment are graphically indicated in Fig. 2. They show the influence of temperature on the respiration of puffer fishes. Ten individuals were submitted to various temperatures as indicated on the graph and the results for each were averaged. It may be observed that the respiratory rhythm (*R*), rate of metabolism (*M*), and quantity of water pumped per minute (*W*) increased progressively with increase in the temperature of the surrounding water.

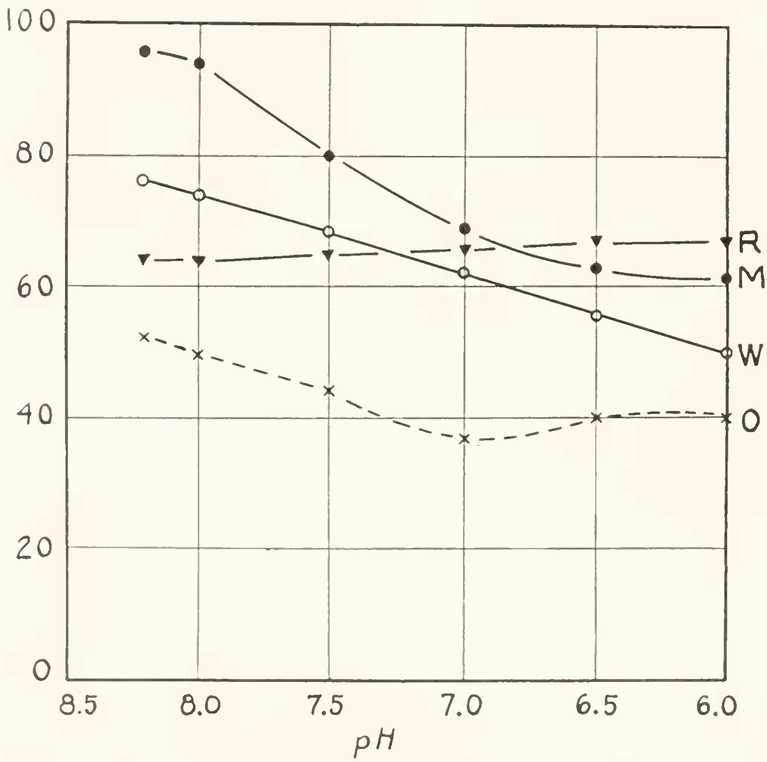

FIG. 3. Graph showing the influence of pH with low CO_2 tensions on respiration of puffer fishes. Scale and legend as in Fig. 2.

The percentage of the dissolved oxygen absorbed from the surrounding water, however, did not increase appreciably. At 20° C., which was approximately the temperature of sea water in the Woods Hole Region at the time these experiments were conducted, puffer fishes had an average rhythm of 80 respirations per minute, pumped 6 liters of water over their gills in an hour, absorbed 45 per cent of the dissolved oxygen from the water, and consumed on the average 62 cc. of oxygen per kilogram of body weight in an hour.

The second experiment shows the effects on the respiration of puffer fishes of varying the hydrogen ion concentration of the surrounding water by the addition of hydrochloric acid to sea water (and subsequent aëration in order to remove excess carbon dioxide). The results ob-

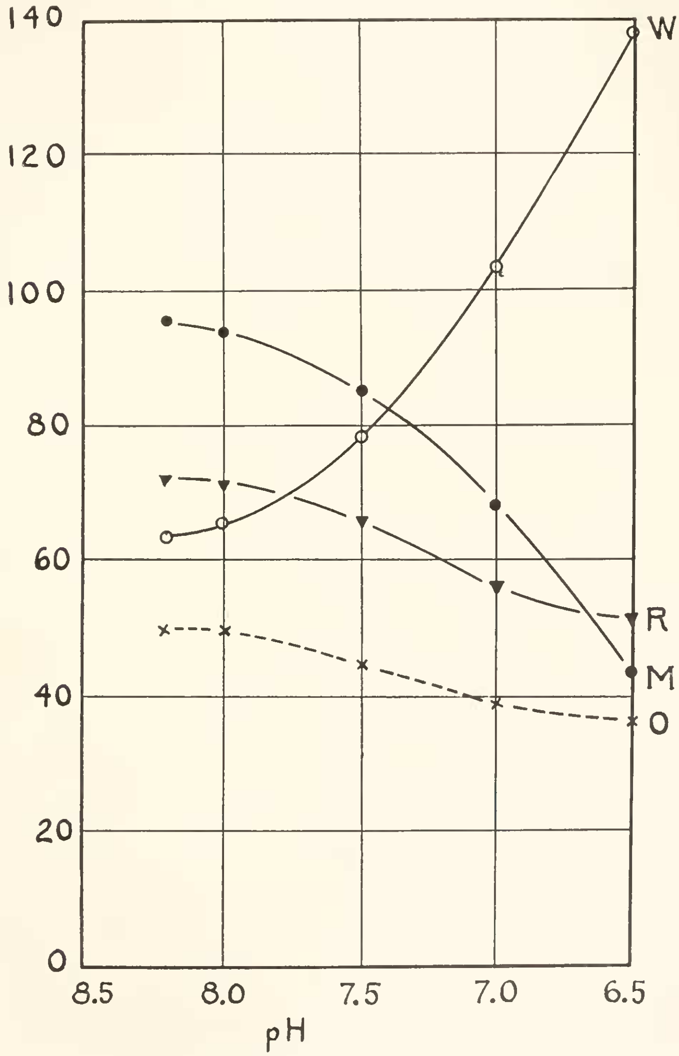

Fig. 4. Graph showing the influence of pH with high CO_2 tensions on respiration of puffer fishes. Scale and legend as in Fig. 2.

tained with six individuals are averaged and summarized graphically in Fig. 3. They show that decreasing pH *per se* apparently inhibits the rate of metabolism (M), and the amount of water pumped by fishes.

The respiratory rhythm (R) is affected slightly. The percentage of oxygen absorbed (O) decreases with increasing acidity.

The third experiment was devised to show how dissolving carbon dioxide would affect respiration as compared with the effect of pH produced by hydrochloric acid in the previous experiment. Figure 4 represents the average results obtained with puffer fishes. These indicate that variations in carbon dioxide concentration expressed in terms of the pH of the water which contains it have a much greater influence on respiration of fishes than variations in pH due to other factors. The quantity of water pumped (W) was markedly accelerated when the sea water approached the acid side of neutrality. The rate of metabolism was greatly inhibited. The respiratory rhythm decreased in rate accordingly. Fishes died when the pH was lowered below 6.5, while in the previous experiment no difficulty was experienced in submitting individuals to a pH of 6.0.

Table I

The percentage of dissolved oxygen absorbed by puffer fish from sea water of varying oxygen tensions at 20° C.

Dissolved Oxygen in cc. Per Liter		Percentage of Dissolved Oxygen Absorbed
Affluent Water	Effluent Water	
4.68	2.16	46
4.00	1.84	46
3.10	1.49	48
2.31	1.10	47
1.14	0.58	45
0.98	0.45	46

The purpose of the fourth experiment was to determine the percentage of oxygen which fishes absorbed at different oxygen tensions. These determinations were made on eight individuals at a constant temperature of 20° C. Dissolved oxygen analyses were made on the affluent water which was being sucked into the mouth of the fishes and on the effluent water which was flowing out of the opercular opening after it had passed the gills. The results obtained with each individual were averaged and are shown in Table I. They indicate that fishes are able to absorb from 45 to 48 per cent of the dissolved oxygen from sea water regardless of wide variation in the tension of the dissolved oxygen in the affluent water.

Discussion

It is evident from the foregoing experiments that the puffer fish is able to pump considerable water over the gills. The quantity of water circulated through the gill clefts and over the gill filaments varies under different circumstances (Fig. 2). When the temperature of the inspired water is increased, the oxygen consumption increases progressively. Concomittantly more water is pumped by the fish. However, the quantity of water which is pumped by a single inspiration and expiration varies but little and remains relatively constant through a wide range of temperature changes. The rate of respirations per minute, on the other hand, shows a parallel increase with that of water pumped and oxygen consumed by the organism. Similarly, the quantity of dissolved oxygen removed does not seem related or influenced by the oxygen consumed, but remains at a fairly constant level. Between temperatures of 12° and 22° C. the variation in the percentage of oxygen removed from the inspired water was between 44 and 45 per cent. It seems, therefore, that the need for an increased quantity of oxygen with increasing temperature is obtained mainly by regulation of the respiratory rhythm and not by the quantity of water pumped on each inspiration or the quantity of oxygen removed from the inspired water.

The gills are apparently a very efficient mechanism through which oxygen is absorbed into the blood. Gill filaments are made of numerous lamellæ, thereby increasing the absorptive surface. Capillaries supply the lamellæ with blood which passes into the general circulation. The outer membrane of the gill filaments is very thin, only a few microns in thickness. Through this membrane dissolved molecular oxygen passes from the sea water into the blood and is there bound by hemoglobin. A small quantity of molecular oxygen will also be found in the blood in the same state as in sea water, *i.e.,* physically dissolved. The oxygen capacity of the blood of puffer fishes has been found to range from 8 to 10 volumes per cent.

When water is pumped into the mouth of the puffer, it is forced out between the branchial arches in such a way that a great proportion of it comes into contact with the gill lamellæ. The gills are flattened and elongated and are fairly close together when water is forced past them. Thus their anatomical arrangement is particularly advantageous. Several factors are to be considered in properly interpreting their function. When a stream of water passes through a branchial cleft its velocity will be greatest in the middle of the stream and least nearest the lamellæ. Relatively more oxygen will consequently be absorbed from the water nearer the lamellæ than from that further away. If oxygen is to be absorbed from the water moving at the higher velocity it must diffuse rather rapidly. The rate of diffusion will depend upon the pressure gradient.

Thus the efficiency of the respiratory mechanism may in a way be determined by comparison of the gas tensions of the affluent and effluent water. Figure 1 shows that at 20° C. puffers pump an average of 6 liters of water per hour over their gills, and that 45 per cent of the dissolved oxygen was removed from affluent water. This indicates a very effective aëration of the gills. Such a conclusion is further substantiated by the fourth experiment, in which the oxygen tension of the affluent water was changed through a series of tensions ranging from 0.9 cc. per liter to 4.8 cc. per liter. It was found that about the same percentage of oxygen was removed regardless of the oxygen tension. The percentage varied only from 45 to 48 per cent. This indicates that the respiratory mechanism of gill aëration is equally efficient over quite a wide range of oxygen tensions.

An interesting point which must be considered in investigations concerned with the respiration of fishes is the absence of any mechanical buffering means such as is present in the alveolar air of air-breathing animals. Mammals particularly have a residual air supply which maintains a fairly constant CO_2 and O_2 tension so that moderate irregularities in breathing only slightly change the gas tensions of the alveolar air. Fishes, however, have their gills directly exposed to water and have nothing comparable to alveolar air tensions. Their gills are directly exposed to the gas tension of the water in which they live. They have apparently no means by which the gas tensions to which their gills are subjected may be altered. Since the amount of CO_2 in sea water is low, the CO_2 tension of the water surrounding the gill filaments would be much lower than the CO_2 tension in the alveolar air of lung-breathing vertebrates. Investigations are now being conducted to determine the CO_2 tension of fishes blood and its rôle in the respiratory function of the blood.

Summary

1. A method is described for studying environmental factors which affect the respiration of fishes.

2. An increase in temperature of water surrounding puffer fishes is followed by increased oxygen consumption by the fishes, a greater quantity of water pumped through the branchial chamber, and a faster respiratory rhythm. The percentage of dissolved oxygen absorbed remains constant at all temperatures observed.

3. Increase in hydrogen ion concentration inhibits oxygen consump-

tion by marine fishes, but addition of CO_2 has a more pronounced effect than addition of HCl at the same pH.

4. The results indicate that marine fishes apparently remove dissolved oxygen from sea water by an efficient mechanism of gill aëration. Fishes absorbed about 46 per cent of dissolved oxygen from sea water at all observed oxygen tensions.

BIBLIOGRAPHY

Gaarder, T., 1918. *Biochem. Zeitschr.*, **89**: 94.
Henze, M., 1910. *Biochem. Zeitschr.*, **26**: 255.
Powers, E. B., 1922. *Jour. Gen. Physiol.*, **4**: 305.
Powers, E. B., 1929. *Ecology*, **10**: 97.
Powers, E. B., 1930. *Am. Nat.*, **64**: 342.
Powers, E. B., and L. M. Shipe, 1928. *Pub. Puget Sound Biol. Sta.*, **5**: 365.
Winterstein, H., 1908. *Pflüger's Arch.*, **125**: 73.